



## Antioxidant Properties of Selected Underutilized Fruit Species of Sri Lanka after Simulated Oral and Gastro-Intestinal Digestion

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### ABSTRACT

**Background:** High consumption of natural antioxidants, particularly phenolic compounds found in fruits and vegetables, makes a significant contribution to plasma antioxidant capacity. The impact of plant phenolics in terms of health benefits is strongly reliant on their level of bioaccessibility and bioavailability.

**Objectives:** The study was conducted to determine the antioxidative properties and bioaccessibility in six underutilized fruit species, namely (*Phyllanthus emblica*, *Elaeocarpus serratulifolius*, *Cynometra cauliflora*, *Aegle marmelos*, *Limonia acidissima* and *Flacoutia indica*).

**Materials & Methods:** The total phenolic content (TPC) of fruit samples was determined after in-vitro simulated oral and gastro-intestinal digestion. The total phenolic content of dialysate and retentates were determined using the Folin-Ciocalteu's test. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and the total antioxidant activity assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS)) were used to assess radical scavenging activity (RSA) and total antioxidant activity (TAA), respectively.

**Results:** The highest TPC (110.33 mg of gallic acid equivalents (GAE)/g on dry weight (DW) basis), the highest DPPH radical scavenging activity as measured by IC<sub>50</sub> value (0.09 mg of dried fruit/mL), and the highest percentage of TAA (90%) were exhibited by *P. emblica* while the *C. cauliflora* recorded the lowest. Among the tested fruit species, *P. emblica* also had maximum bioaccessibility of total polyphenols (83.66 %) and *C. cauliflora* had the lowest (40.16%).

**Conclusions:** *Phyllanthus emblica* had the highest TAA and RSA of the tested fruit species while lowest was recorded in *C. cauliflora*. The TPC, TAA and RSA of all fruit species increased after enzymatic digestion. Further *in vitro* and *in vivo* studies should be conducted to assess the bioaccessibility and bioavailability of individual phenolic compounds.

## INTRODUCTION

Epidemiological studies have reported a link between high intake of fruits and vegetables and a reduction in diseases caused by oxidative stress, which is specifically linked to the antioxidant property of phytochemicals (Souza *et al.*, 2015). High consumption of natural antioxidants, particularly phenolic compounds found in fruits and vegetables, makes a significant contribution to plasma antioxidant capacity, and these constituents have been shown to reduce the damage caused by oxidative stress (Lie-Fen *et al.*, 2005). However, the impact of plant phenolics in terms of health benefits is strongly reliant on their level of bioaccessibility and bioavailability (Shahidi and Peng, 2018). Thus, many *in vivo* and *in vitro* models are used to investigate the bioaccessibility and bioavailability of phenolics. *In vitro* digestion models are widely used because they are less expensive, easier to operate, have a higher efficiency, and are more reproducible than *in vivo* models (Minekus *et al.*, 2014). The static simulated digestion model is a simple and widely used *in vitro* method for screening the bioaccessibility of polyphenolic compounds (Carbonell-Capella *et al.*, 2014).

The ability to determine the biological activity of dietary components requires knowledge of absorption in the digestive system (Shahidi and Peng, 2018). The bioaccessibility refers to the portion of bioactive compounds absorbed from the intestine that are released from the food matrix after digestion (Shahidi and Peng, 2018), whereas the bioavailable fraction is the fraction of parent compound found in the systemic circulation or at the target site after absorption (Ștefănescu *et al.*, 2019). The bioaccessibility was the primary factor limiting the bioavailability, making it important to assess (Campos-Vega *et al.*, 2015).

Sri Lanka is a tropical country with a diverse plant population, including over

230 fruit species from 57 plant families (Pushpakumara *et al.*, 2007). Aside from commonly consumed fruit crops such as banana, pineapple, papaya, mango, avocado, and rambutan, there are a myriad of underutilized fruit species that grow naturally in diverse regions around Sri Lanka and contribute to traditional recipes (Rajapaksha, 2007).

Extensive research has been done recently on the antioxidant qualities of underutilized fruit species. Silva and Sirasa (2018) evaluated the antioxidant capacities of 18 underused fruit species with those of fruit species that are marketed commonly (mango and banana). The findings showed that some rarely consumed fruit species contained more antioxidants than more widely consumed fruits. The antioxidant efficacies of 21 underutilized Sri Lankan fruit species were examined by Mallawaarachchi *et al.* in 2021, suggesting their potential as natural antioxidant sources. Despite the *in vitro* antioxidant properties of underutilized fruits, reports on the antioxidant properties of bioaccessible phenolics derived from them are scarce. In this study, the antioxidant properties of bioaccessible phenolics of chosen underutilized fruit species were assessed after simulated oral and gastro-intestinal digestion.

## MATERIALS & METHODS

### Fruit Samples

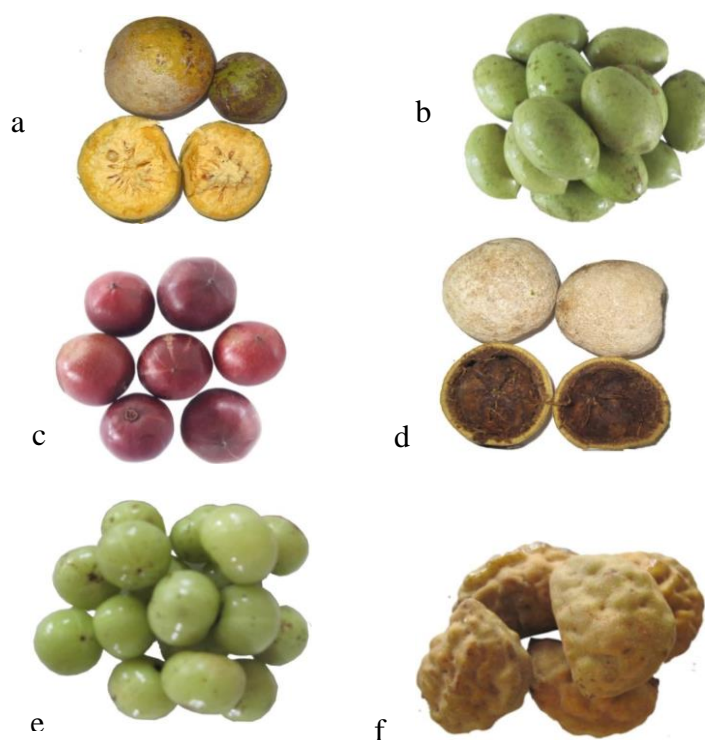
Healthy and ripe fruits of six fruit species namely, *Phyllanthus emblica* L., *Elaeocarpus sirratus* Linn, *Cynometra cauliflora* Linn., *Aegle marmelos* L. Correa, *Limonia acidissima* L. and *Flacoutia indica* (Burm. f.) (Plate 1) were collected from home gardens in the upcountry intermediate zone (IU3) of Sri Lanka during the peak production period of 2018. Cold transport was used to transport the collected fruit samples to the analytical laboratory at the Regional Agriculture Research and Development Centre in Bandarawela. They were then inspected for

defects, washed, and drained at room temperature before being authenticated (Rajapaksha, 1998) and photographed. The color of the fruits were recorded, and a hundred grams of edible portions of the fruits from each fruit species were taken and homogenized. The *in vitro* digestion study employed five grams of each homogenate in triplicate.

### Chemicals and reagents

The analytical grades of gallic acid, methanol, Folin-Ciocalteu's phenol reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 2,2-

azinobis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH), hydro chloric acid (99%), magnesium chloride hexa-hydrate, potassium chloride, potassium di-hydrate phosphate, sodium bi-carbonate, ammonium carbonate, calcium chloride di-hydrate, sodium hydroxide, piperazine NN'-bis (2-ethane-sulfonic acid) (PIPES),  $\alpha$ -amylase from human saliva (300-1500 units/mg, Type XIII-A, lyophilized powder), pancreatin from porcine pancreas, pepsin from porcine gastric mucosa  $\geq 500$  units/mg, porcine bile extract were purchased from Sigma, USA.



**Plate 1.** Selected fruit species.

(a-*Aegle marmelos*, b- *Elaeocarpus serratus*, c- *Flacourtia indica*, d- *Limonia acidissima*, e- *Phyllanthus embilica*, f- *Cynometra cauliflora*)

### Preparation of digestion fluids

Table 1 summarizes the ionic solution proportions used to prepare simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) (Minekus et al., 2014). To obtain final ionic concentrations and to adjust appropriate pH in corresponding digestion fluids, volumed up with distilled water and 1 M NaOH and

6 M HCl were used, respectively. Salivary  $\alpha$ -amylase (1500 units/mL), porcine pepsin (2000 units/mL) and porcine pancreatin (800 units/mL) solution were prepared separately in electrolyte solutions SSF, SGF, and SIF (Minekus et al., 2014). In 250 mL of SIF, three grams of porcine bile extract were dissolved (Akillioglu and Karakaya, 2010).

**Table 1.** Concentrations and compositions of ionic solutions (in 500 mL)

Constituent	Concentration of ionic (stock) solutions (mol/L)	SSF at pH 7	SGF at pH 3	SIF at pH 7
		Stock volume (mL)	Stock volume (mL)	Stock volume (mL)
KH <sub>2</sub> PO <sub>4</sub>	0.5	3.70	0.90	0.80
KCl	0.5	15.10	6.90	6.80
NaHCO <sub>3</sub>	1.0	6.80	12.50	42.50
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	0.5	0.06	0.50	-
NaCl	2.0	-	-	9.60
MgCl <sub>2</sub> 6H <sub>2</sub> O	0.15	0.50	0.40	1.1
CaCl <sub>2</sub> 2H <sub>2</sub> O	0.3	25 $\mu$ L <sup>†</sup>	5 $\mu$ L <sup>†</sup>	40 $\mu$ L <sup>†</sup>

<sup>†</sup>Added to the sample at the beginning of digestion

SSF = Simulated salivary fluid, SGF= Simulated gastric fluid, SIF = Simulated intestinal fluid

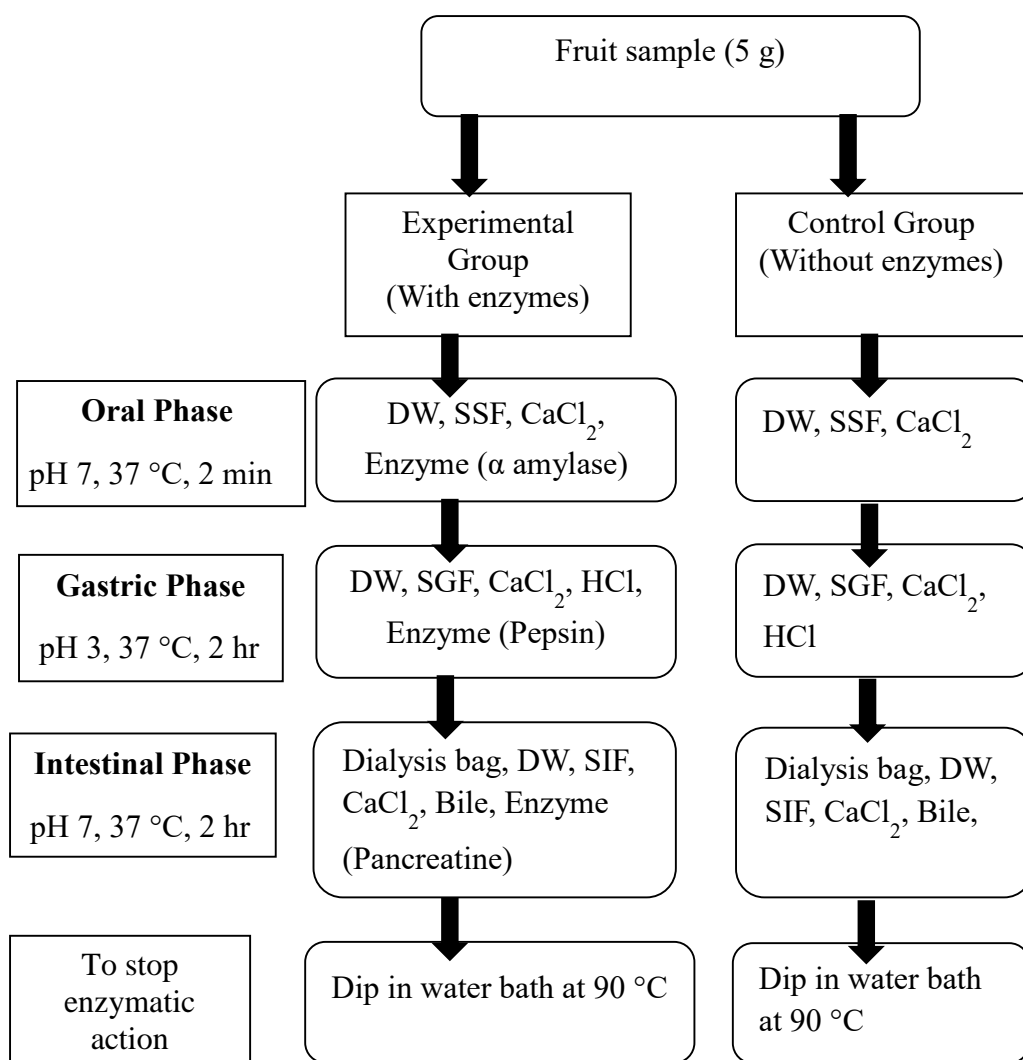
### Simulated digestion

Figure 1 depicts the detailed procedure. The simulated digestion was carried out using combined methods described by Akillioglu and Karakaya (2010) and Minekus *et al.* (2014), with some modifications. Samples were prepared using enzymes (experimental group) and without enzymes (control group). Then, the digestion was carried out in 100 mL polystyrene tubes dipped in a shaking water bath at 37 °C. Following oral and gastric digestion, dialysis tubes (typical molecular cut off point of 14 000) with PIPE buffer were dipped in each polystyrene tube to proceed to the intestinal digestion phase. The pH of the digestion media was adjusted accordingly in each phase. Following incubation, polystyrene tubes containing samples and dialysis tubing were immersed in a water bath at 90 °C for

10 minutes (Hollebeeck *et al.*, 2013). The dialysis bag was then removed and washed with distilled water. Supernatants of retentate and dialysate were collected after centrifugation to be used in the respective assays.

### Determination of total phenolic content

The total phenol content was determined using Folin-Ciocalteu colorimetric assay (Yu *et al.*, 2002). In brief, 20  $\mu$ L of sample (supernatants of dialysate/retentate) was vortexed with 100  $\mu$ L and 1.58 mL of 2 N Folin-Ciocalteu's reagent and distilled water, respectively, and allowed to stand at room temperature for 8 min, before adding 300  $\mu$ L of 0.7 M sodium carbonate and incubating for 30 min at room temperature, absorbance was measured at 765 nm by using Helios Omega-UV-VIS spectrophotometer. The samples were examined in triplicate.



**Figure 1.** Schematic diagram depicting simulated digestion steps

(DW= Distilled water, SSF= Simulated salivary fluid, SGF= Simulated gastric fluid, SIF= Simulated intestinal fluid)

The TPC was calculated using the gallic acid standard curve ( $y= 0.0007x$ ,  $R^2=0.9999$ ) and expressed in milligrams of gallic acid equivalents (GAE) per gram of dried fruit.

#### **Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity**

The DPPH radical scavenging assay (Su *et al.*, 2007) was used to assess the radical scavenging activity (RSA) of the dialysates and retentates. For that, 0.02 – 0.20 mL of each dialysate and retentate were used to prepare six different concentrations, and

methanol was added to obtain the final 2.0 mL of reaction medium with 1.8 mL of 0.1M methanolic DPPH solution. The results were expressed as  $IC_{50}$  values, which denote the concentration of extract required to scavenge 50% of the DPPH radical in the reaction medium, as determined by the percentage of color disappearance of the DPPH solution vs. concentration plot.

#### **Determination of total antioxidant activity**

Total antioxidant activity (TAA) assay was used to determine the TAA as described by Zhou and Yu (2004). To perform the TAA

assay 1.96 mL of stock (2.5 mM ABTS in PBS buffer at pH 7.4 and 2 mM AAPH in PBS buffer at pH 7.4 in 1:1 ratio) was mixed with 0.04 mL of samples (retentate and dialysate), and absorbance was measured at 734 nm on the 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> minute. Over a six-minute period, the results were expressed as a percentage inhibition of ABTS radical cation in terms of RSA. The following equation was used to compute the RSA.

$$\text{RSA\%} = \{1 - (A_{\text{sample}}/A_{\text{control}})\} * 100$$

$A_{\text{sample}}$  = Absorbance of the sample and

$A_{\text{control}}$  = Absorbance of the control

### Determination of bioaccessibility of phenolic compounds

The bioaccessibility is the proportion of bioactive compounds that are liberated from the food matrix during digestion and absorbed from the intestine (Shahidi and Peng, 2018) and calculated as follows.

$$\text{Bioaccessibility (\%)} = \frac{\text{TPC in dialysate}}{\text{TPC in dialysate} + \text{TPC in retentate}} \times 100$$

TPC = Total phenolic content

### Statistical analysis

SAS 9.1 statistical software was used to analyze quantitative data on bioaccessible total phenolic compounds, DPPH radical scavenging activity, and total antioxidant activity of retentate and dialysate. Analysis of variance and least significant difference tests were used to identify mean differences at 5% probability.

## RESULTS

### Bioaccessibility, total phenolic content and antioxidant activity of dialysates and retentates

The bioaccessibility of phenolic compounds, total phenolic content (TPC, Table 2), DPPH radical scavenging activity (IC<sub>50</sub>, Figure 2) and total antioxidant activity (TAA, Figure 3) of the supernatants of dialysate and retentate obtained after oral

and gastro-intestinal digestion were all determined. After oral and gastro-intestinal digestion, the bioaccessibility of total phenols in selected fruit species ranged from 40% in *C. cauliflora* to 84% in *P. emblica* (Table 2), *A. marmelos* had the second highest bioaccessibility, while the percentage absorbed by *F. indica* and *L. acidissima* was not significant. After gastro-intestinal digestion, nearly 50% of total phenols (TP) of *E. serratus* were bioaccessible. The dialysate and retentate obtained after enzymatic digestion of *P. emblica*, possessed the highest sum of total phenols while *C. cauliflora* possessed the lowest. The sums of the control group's TP values ranged from 5.69 mg GAE/g dried fruit in *C. cauliflora* to 106.54 mg GAE/g of dried fruit in *P. emblica*. Meanwhile, the TPC of dialysate and retentate obtained from the control group of all fruit species that were performed without enzymatic digestion were significantly lower than those obtained from the experimental group of each fruit species.

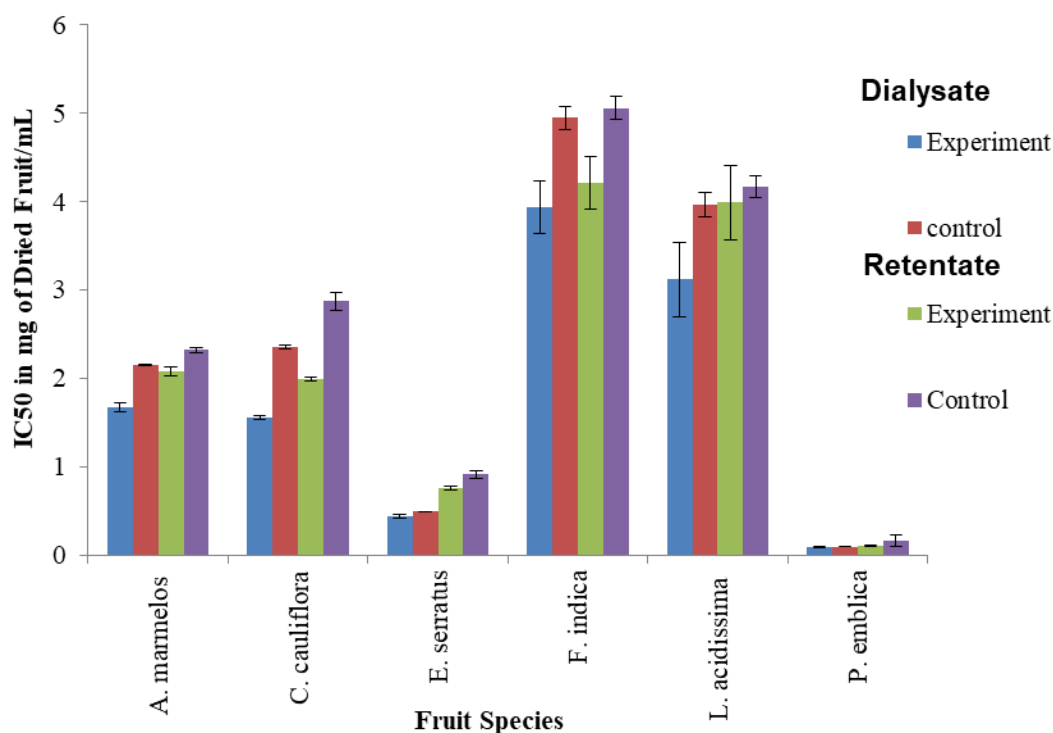
### DPPH radical scavenging activity of dialysates and retentates

Figure 2 depicts the radical scavenging activity (RSA) of each dialysate and retentate. Lower IC<sub>50</sub> values indicate the higher RSA, thus the dialysates of experimental group had the lowest IC<sub>50</sub> values among four groups in each fruit species, indicating that RSA was increased after enzymatic digestion. Zhou *et al.* (2016) discovered the same results in the dialysate obtained after *in vitro* gastrointestinal digestion of elderberry. *P. emblica* possessed the lowest IC<sub>50</sub> value, 0.09 mg of dried fruit/mL in both experimental and control dialysates, and 0.10 and 0.16 mg of dried fruit/mL in experimental and control retentates, respectively, denoting the highest RSA and not being significantly different at  $p < 0.05$  (Figure 2). *F. indica* had the highest IC<sub>50</sub> values for all four groups exhibiting lowest RSA (3.94 and 4.95 mg of dried fruit/mL for the dialysates of the experimental and

control groups, 4.21 and 5.06 mg of dried fruit/mL for the retentates of the experimental and control groups, respectively) (Figure 2). The second highest  $IC_{50}$  values for all four groups were obtained by *L. acidissima*.

While *E. serratus* had the second lowest  $IC_{50}$  with 0.44 mg of dried fruit/mL in

experimental group dialysate (Figure 2). *C. cauliflora*, which had the lowest bioaccessibility, had significantly lower  $IC_{50}$  values than *A. marmelos*, *F. indica* and *L. acidissima* for the dialysate of experimental group.



**Figure 2.**  $IC_{50}$  values in milligrams of dried fruit/mL of dialysates and retentates of experimental and control groups

### Total antioxidant activity of dialysates and retentates

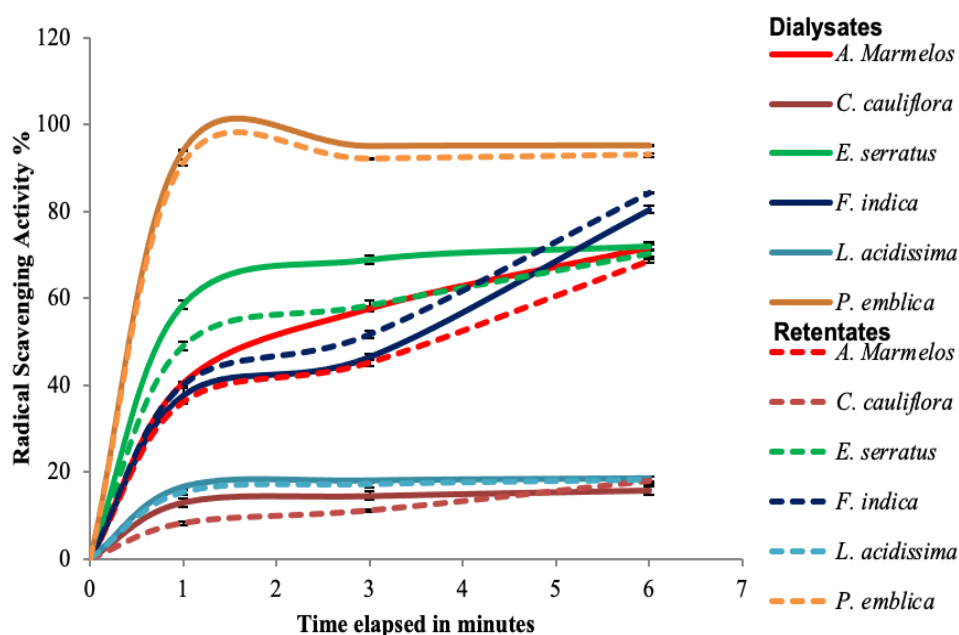
The ABTS assay was used to assess total antioxidant activity (TAA) based on RSA, which indicates the ability of dialysates and retentates to reduce the color of deep blue green ABTS radical cation stock solution. The TAA as RSA of each fruit species is depicted in Figure 3 over a six-minute reaction time. The dialysate and retentate of the experimental group of *P. emblica* demonstrated the highest TAA, achieving more than 90% RSA within the first minute

and eventually reaching 95% and 93% RSA, respectively (Figure 3a).

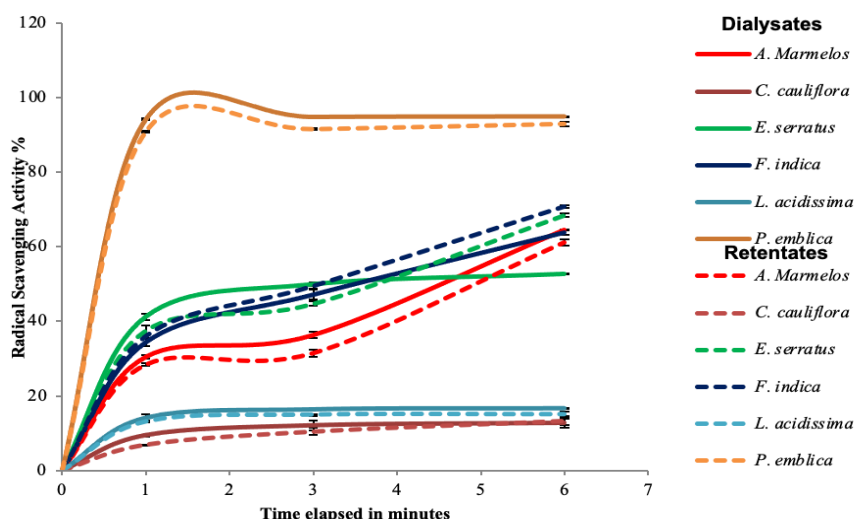
The second highest RSA was found in *F. indica* at sixth min, in which the retentate (84.4%) had a significantly higher RSA than the dialysate (80.5%). After six minutes, the RSA of *A. marmelos* (71.8%) and *E. serratus* (71.9%) dialysates were not significant. Although *A. marmelos* exhibited approximately 40% RSA, *E. serratus* exhibits 58% RSA within the first minute. The RSA values of *C. cauliflora* and *L. acidissima* dialysates and retentates were not-significant and had the lowest

RSA of any fruit species tested. Furthermore, the reaction of *F. indica* in both dialysate and retentate with ABTS cation radicals were increased rapidly after the 3<sup>rd</sup> minute, whereas other fruit species, with the exception of *A. marmelos*, remained constant (Figure 3a). Figure 3b

depicts the RSA of undigested (control) groups, revealed significantly lower RSA values for dialysates and retentates than in experimental group for all fruit species except *P. emblica*.



3a. Radical scavenging activity of dialysates and retentates of fruit pulp after oral and gastrointestinal digestion



3b. Radical scavenging activity of dialysates and retentates of undigested fruit pulp

Figure 3. Radical scavenging activity of dialysates and retentates



**Table 2.** Total phenolic content of dialysates and retentates and bioaccessibility of total phenolics of selected fruit species

Fruit Species	Sinhala Name	Bioaccessibility of total phenolics (%) of experimental group	†TPC (mg GAE/g of DW) ‡					
			Dialysate		Retentate		Total	
			Experimental group	Control	Experimental group	Control	Experimental group	Control
<i>A. marmelos</i>	<i>Beli</i>	74.08 ± 2.36 <sup>b</sup>	28.69 ± 0.32 <sup>bA</sup>	18.62 ± 0.02 <sup>bB</sup>	10.04 ± 0.41 <sup>bC</sup>	4.84 ± 0.16 <sup>bD</sup>	38.73 ± 2.49 <sup>bE</sup>	23.46 ± 1.59 <sup>bF</sup>
<i>C. cauliflora</i>	<i>Nami-nam</i>	40.16 ± 4.58 <sup>e</sup>	4.11 ± 0.13 <sup>dA</sup>	3.17 ± 0.04 <sup>fB</sup>	6.12 ± 0.24 <sup>cC</sup>	2.52 ± 0.43 <sup>dD</sup>	10.23 ± 1.07 <sup>eE</sup>	5.69 ± 0.64 <sup>eF</sup>
<i>E. serratus</i>	<i>Weralu</i>	49.25 ± 1.09 <sup>d</sup>	6.25 ± 0.96 <sup>cdA</sup>	4.61 ± 0.08 <sup>eB</sup>	6.44 ± 0.87 <sup>cC</sup>	3.98 ± 0.71 <sup>cdD</sup>	12.69 ± 0.98 <sup>dE</sup>	8.59 ± 0.47 <sup>dF</sup>
<i>F. indica</i>	<i>Uguressa</i>	64.65 ± 2.79 <sup>c</sup>	8.78 ± 0.12 <sup>cA</sup>	6.72 ± 0.01 <sup>dB</sup>	4.80 ± 0.08 <sup>dC</sup>	2.56 ± 0.29 <sup>dD</sup>	13.58 ± 0.76 <sup>cdE</sup>	9.28 ± 0.81 <sup>cdF</sup>
<i>L. acidissima</i>	<i>Diwul</i>	60.21 ± 3.02 <sup>c</sup>	8.91 ± 0.09 <sup>cA</sup>	7.51 ± 0.09 <sup>cB</sup>	5.89 ± 0.17 <sup>cdC</sup>	2.39 ± 0.14 <sup>dD</sup>	14.80 ± 1.03 <sup>ceE</sup>	9.90 ± 0.94 <sup>ceF</sup>
<i>P. emblica</i>	<i>Nelli</i>	83.66 ± 2.65 <sup>a</sup>	110.33 ± 1.79 <sup>aA</sup>	92.30 ± 1.28 <sup>aB</sup>	21.55 ± 1.26 <sup>aC</sup>	14.24 ± 0.93 <sup>aD</sup>	131.88 ± 4.21 <sup>aE</sup>	106.54 ± 2.54 <sup>aF</sup>

† Values with different lower-case letters in each column are significantly different at  $p < 0.05$

‡ Values with different upper-case letters in each row of each variable are significantly different at  $p < 0.05$

Data are presented as Mean ± Standard error, GAE = Gallic acid equivalents. DW = Dry weight basis, TPC = Total phenolic content

## DISCUSSION

### Bioaccessibility and total phenolic content of dialysates and retentates

Semi-permeable cellulose membranes (dialysis tubes) were used to simulate the intestine's gut epithelial cell layer and to allow for the free diffusion of dietary phenolics. The cut-off molecular weight of dialysis membrane was the factor that determines the penetration. Phenolics with a molecular weight greater than the cut-off point (14 000) remain in the digestive part (retentate), whereas low molecular weight compounds migrate into the dialysate. To achieve the same *in-vivo* ionic concentration, electrolyte stock solutions and digestion fluids were prepared (Minekus *et al.*, 2014). After the digestion process, the polystyrene tubes with dialysis bags were dipped in a water bath at 90 °C for 10 minutes to stop further enzymatic reactions (Hollebeeck *et al.*, 2013).

The total phenol content (TPC) of dialysate and retentate from the experimental group of each fruit species were considerably higher than those from the control group of all fruit species, which did not undergo enzymatic digestion. This increase in TPC in the experimental group could be attributed to the release of bound phenolics from macromolecules such as protein and carbohydrates during enzymatic digestion. Akillioglu and Karakaya (2010) obtained the same results in common and pinto beans after *in-vitro* digestion.

The TPC of water extracts of ripe fruits before digestion were 21.46, 4.59, 2.19, 14.35, 6.25, and 103.75 mg GAE/g DW in *A. marmelos*, *C. cauliflora*, *E. serratus*, *F. indica*, *L. acidissima* and *P. emblica*, respectively (Mallawaarachchi *et al.*, 2021), which were lower than the values obtained after *in-vitro* digestion. Based on those findings of the previous study of the authors, the amount of TP in fruit species increased after *in-vitro* digestion by 80.5% in *A. marmelos*, 122.9% in *C. cauliflora*, 479.5% in *E. serratus*, 136.8% in *L.*

*acidissima* and 27% in *P. emblica* with *F. indica* exhibiting the same level of TP in both raw and digested sections. This could be explained by the extraction solvents' limited ability to extract polyphenolic compounds from the food matrix, as well as pH changes during digestion and the action of digestive enzymes, which may facilitate polyphenol release.

### Antioxidant activity of dialysates and retentates

#### *DPPH radical scavenging activity of dialysates and retentates*

The radical scavenging activity of dialysates after enzymatic digestion was significantly greater than that of the undigested part (control) (Figure 2). This could be related to the release of bound phenolics from macromolecules, which enhances their solubility, as well as the hydrolysis of conjugate polyphenols as a result of enzymatic activity in the oral, gastric, and intestinal phases, as well as the effect of low pH in the gastric phase.

#### *Total antioxidant activity of dialysates and retentates*

Previous research has found that the fruits of *P. emblica* possess a high concentration of vitamin C (523 mg/100 g) (Mallawaarachchi *et al.*, 2021). If the composition of vitamin C in the food matrix is high, the bioaccessibility of vitamin C is high (Brandon *et al.*, 2014), which contributes between 47% and 70% of the fruit's antioxidant activity (Charoenteeraboon *et al.*, 2010). Thus, it may account for its higher TAA. The fact that the retentate of *F. indica* at sixth minute had a much higher RSA than the dialysate may be related to the presence of polyphenolic chemicals that are larger than the molecular cut-off points of the dialysis tubing. Although the RSA of *A. marmelos* and *E. serratus* dialysates were not significant after six minutes, *A. marmelos* had a lower RSA than *E. serratus* within the first minute, indicating that antioxidant

compounds in *E. serratus* react with ABTS cation radicals more quickly than antioxidant compounds in *A. marmelos*.

Polyphenol absorption and metabolism are influenced by physicochemical properties such as solubility, molecular weight, and degree of polymerization and conjugation. Both free (aglycones) and conjugated (glycosides) forms of polyphenols are found in dietary matrix, with the former being quickly absorbed from the small intestine and the latter requiring enzymatic hydrolysis to be absorbed (Ștefănescu *et al.*, 2019). As a result, the undigested portion of this experiment had lower TP, RSA and TAA values than the digested group. The presence of high tannin content was attributed to high antioxidant efficacy of *P. emblica* (Charoenteeraboon *et al.*, 2010). Tannins have a strong affinity for proteins and dietary fiber (Campos-Vega *et al.*, 2015), and the action of pepsin in the gastric phase causes these protein-bound portions to be released, increasing antioxidant activity and absorption. This could be the reason for the higher TAA of dialysate and retentate of *P. emblica*. Campos-Vega *et al.* (2015) discovered that tannin boosts antioxidant activity in the absorbed fraction during *in-vitro* simulated digestion of coffee.

Phenols with a high degree of polymerization are fermented and degraded by colonic bacteria or excreted, whereas phenolics with a low degree of polymerization are easily absorbed in the stomach and small intestine (Shaidi and Peng, 2018). As a result, after gastrointestinal digestion, food containing heavily polymerized phenols may have low bioaccessibility. After gastric digestion of apple, 65 percent of phenolics and flavonoids were released, and the antioxidant capacity of dialysable antioxidant was 57% lower than in fresh apples (Bouayed *et al.*, 2011); this suggested that some polyphenols bound to macromolecules are non-dialysable, and some aglycones remained in the digesta.

The bioavailability and bioaccessibility of each phenolic differed; gallic acid, catechin, flavanone, and quercetin glucosides are well-absorbed, whereas proanthocyanidins and anthocyanins are poorly absorbed (Manach *et al.*, 2005). As a result, fruit extracts with high anthocyanin contents may not be more bioaccessible.

## CONCLUSIONS

*Phylanthus emblica* had the highest bioaccessibility of TPC among the selected fruit species (83.66%), followed by *A. marmelos* > *F. indica* = *L. acidissima* > *E. serratus* > *C. cauliflora*. After simulated oral and gastrointestinal digestion, antioxidant efficacy increased in all fruit species. *Phylanthus emblica* had the highest TAA and RSA of the tested fruit species while lowest was recorded in *C. cauliflora*. Further *in vitro* and *in vivo* studies should be conducted to assess the bioaccessibility and bioavailability of individual phenolic compounds.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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